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Phylogeny and biogeography of the Canarian *Solanum vespertilio* and *S. lidii* (Solanaceae)

by

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Abstract

The endemic and rare *Solanum vespertilio* and *S. lidii* from the Canary Islands are notable in being andromonoecious, self compatible, highly heterandrous (with short >reward= anthers and one very long >pollination= anther), and bearing strongly zygomorphic corollas. *Solanum vespertilio* flowers are also tetramerous, and some display enantiostyly. Given their island distribution, uncommon for solanums, and many distinctive reproductive features, the phylogenetic position is of interest. With new molecular data from the chloroplast *trn*T-F region and the nuclear granule-bound starch synthase gene (GBSSI or *waxy*), we confirm the hypothesis that these two species are phylogenetically associated with *Solanum* lineages from Africa, rather than with previously suggested Mexican species. The phylogeny facilitates understanding the evolution of the unique suite of reproductive characteristics in these island endemics.

Keywords: subgenus Leptostemonum; *Solanum vespertilio*; *Solanum lidii*; *trn*T-F region; granule-bound starch synthase gene; andromonoecy; heterandry; enantiostyly.

Resumen

Las endémicas y raras especies *Solanum vespertilio* y *S. lidii* de las Islas Canarias son notables por ser andromonoicas, autocompatibles, altamente heterándricas (con anteras cortas para recompensa y una muy larga para polinización) y por tener corolas fuertemente zigomórficas. Las flores de *Solanum vespertilio* son, además, tetrámeras y muestran enantiostilia. Dada su distribución insular, poco común para *Solanum*, y sus distintivas características reproductivas, su posición filogenética es de interés. Con nuevos datos moleculares de la región *trn*T-F del ADN del cloroplasto y del gen nuclear de la enzima almidón sintasa unida al gránulo (GBSSI o *waxy*), confirmamos la hipótesis de que estas dos especies están filogenéticamente asociadas con los linajes geográficamente próximos de *Solanum* africanos, más que con especies mexicanas previamente sugeridas. La filogenia facilita la comprensión de la evolución del conjunto singular de características reproductivas de estas especies endémicas de islas.

Palabras clave: subgénero Leptostemonum; *Solanum vespertilio*; *Solanum lidii*; región *trn*T-F; gen de la enzima almidón sintasa unida al gránulo; andromonoecia; heterandria; enantiostilia.

Introduction

Oceanic islands provide special natural laboratories for studying the processes and results of evolution (e.g., Carlquist, 1974; Adsersen, 1995; Stuessy & Ono, 1998). These islands are particularly attractive because their geographical borders (inhospitable salt water) and temporal boundaries (datable volcanic eruptions) are precise. When the arrival of species can be dated rather exactly, differences from presumed progenitors can be assessed in a way not usually possible with continental organisms. If there is also a reliable phylogeny of the island species relative to their continental relatives, then speculations about the direction of the evolution is possible (Carine, 2005).

The Canary Islands (Spain) range from 100-250 km off the western coast of Africa at an average of 281 N latitude in the Atlantic Ocean. This archipelago consists of seven major islands and is of volcanic origin. Volcanism is still active, including El Teide, Spain=s highest mountain. Some of the islands have a recent quaternary origin (1 m. y.), and others are old (up to 40 m. y.), with some estimates putting the oldest at 70-80 m. y. (Coello & al., 1992; Anguita & Hernán, 2000).

The Solanaceae is poorly represented on islands. In the Canary Islands, there are only four endemic

species, all from two genera of the tribe Solaneae (Bramwell & Bramwell, 2001). *Withania* Pauq., a genus up to 20 spp. worldwide (Hunziker, 2001), is represented by the endemic *W. aristata*, a common species found at low elevations fringing all the islands in the archipelago (Bramwell & Bramwell, 2001) and is also notable reproductively (constituting another case of cryptic dioecy in the Solanaceae; Anderson & al., 2006). The remaining three endemic species belong to the highly variable, cosmopolitan, and economically important genus *Solanum* L. (Nee, 1999). One of these (*S. nava* Webb & Berthel. of section *Normania*) has been the subject of considerable previous work (e.g., Francisco-Ortega & al., 1993). It is phylogenetically distinct from the species of interest herein (Bohs & Olmstead, 2001), and is not pursued further.

The species of particular emphasis herein are *S. lidii* and *S. vespertilio* (Fig. 1). The former is endemic to Gran Canaria Island and is represented by only a few, small populations. *Solanum vespertilio* is represented by several populations, some of good size, on Tenerife (*S. vespertilio* subsp. *vespertilio*), and also by a subspecies restricted to Gran Canaria Island (*S. vespertilio* subsp. *doramae* Marrero and González-Martín) which, based on historical records was apparently always rare and today consists of only one population of 2-3 known plants (Marrero & González-Martín, 1998; Bañares & al., 2004). Subspecies *doramae* is delimited by a suite of quantitative characters, such as smaller fruits and leaves, darker corolla, longer calyx lobes, and less undulated petal margins (Marrero & González-Martín, 1998); it is difficult to judge its systematic validity, due to the few available individuals and the nature of the characters used. Unfortunately, this taxon is also so rare that material was not available for the molecular studies.

Both *S. vespertilio* and *S. lidii* are included in the "spiny solanums", i.e., *Solanum* subgenus *Leptostemonum* (Dunal) Bitter, and have a remarkable combination of flower morphological characters (Anderson & al., 2005; Dupont & Olesen, 2006) consisting of strongly zygomorphic corollas (Fig. 1B, D), somewhat uncommon in the genus *Solanum* (Whalen, 1984), and heteromorphic anthers (one long anther and 3-4 anthers of normal length; Fig. 1B, D), i.e.,

Fig. 1. *Solanum vespertilio* subsp. *vespertilio*. **a,** plant on Tenerife Island; **b,** Hermaphroditic flower. *Solanum lidii*. **c,** plant on Gran Canaria Island; **d,** Hermaphroditic flower. Scale = 40 cm for A, 0.95 cm for B, 30 cm for C, 0.9 cm for D.

heterandry, a phenomenon represented in a few species across the genus (Lester & al., 1999). In addition, both species are andromonoecious (Anderson & al., 2005; Dupont & Olesen, 2006) and self compatible (Anderson & al., 2005). Andromonoecy is widely distributed in *Solanum*, though largely limited to the "spiny solanums" (Whalen & Costich, 1986). Self compatibility is also widely distributed in the genus, particularly in the "spiny solanums" (Whalen & Anderson, 1981). A significant percentage of flowers of *S. vespertilio* are enantiostylous (Fig. 1B). Enantiostyly, i.e. the deflection of the style either to the left (left-styled) or right (right-styled) side of the floral axis, is rare in the angiosperms (Jesson & Barrett, 2003; Jesson & al., 2003). However, it is found in *Solanum*, in particular *S. rostratum*, one of the exemplars for this phenomenon (and in some other members of section *Androceras*: e.g., Todd, 1882; Bowers, 1975; Whalen, 1979; Jesson & Barrett, 2005). *Solanum vespertilio* is also distinctive in bearing only four anthers (one long and three short) instead of the typical five, and concomitantly, four calyx and corolla lobes (Fig. 1B; Anderson & al., 2005). The four-merousness of *S. vespertilio* is also not unique in *Solanum* (Whalen, 1984; Jaeger, 1985), but is highly uncommon. These reproductive features are part of a comprehensive ongoing study of the reproductive, pollination and population biology of this Canarian species pair (Anderson & al., 2005).

Given the plasticity of some of the taxonomic characters of *Solanum*, and the huge size of the genus, taxonomic treatments and phylogenies pose a challenge. For instance, there have been two or three different classifications for this Canary Island species pair. These taxonomic treatments present different geographical scenarios. One treatment links *S. vespertilio* and *S. lidii* with Mexican species based on shared enantiostyly and zygomorphy (Whalen, 1984), and another links the Canarian species with the geographically closer African taxa, this latter treatment based on seed epidermis analyses (Lester & al., 1999).

The most effective analysis of the adaptation and evolution of reproductive systems depends fundamentally on a sound phylogeny. Accordingly, we used several morphologically-independent molecular characters, i.e., data from the chloroplast *trn*T-L and *trn*T-F intergeneric spacer regions (Taberlet & al., 1991) and the granule-bound starch synthase nuclear (GBSSI or *waxy*) gene (van der Leij & al., 1991; Mason-Gamer & al., 1998) to clarify the phylogenic associations of the two *Solanum* species. This analysis also provides the foundation for appropriate study of biogeographic radiation of the genus as well.

Material and methods

Sampling

In addition to the two solanums in question (*S. lidii* and *S. vespertilio* subsp. *vespertilio*), we also included another 19 taxa from *Solanum* subgenus *Leptostemonum* (Table 1), in particular from Africa and Mexico, that share some reproductive features expressed in the Canarian species. Thus, we include such species as *S. tridynamum*, a heterandrous and andromonoecious species from Mexico, *S. citrullifolium* and *S. rostratum*, two heterandrous, enantiostylous taxa native to Mexico and the southwestern United States, all with zygomorphic flowers, and *S. myoxotrichum*, with 3 to 5 merous flowers, often 4-merous, from Madagascar. In addition, two non-spiny solanums, *S. trisectum* (a Madeiran species with zygomorphic, heterandrous flowers) and *S. herculeum* (from southern Spain and northern Africa, with slightly zygomorphic flowers), as well as species from other subgenera and regions of the world, were included in the analysis for geographic and morphological context. These were selected from a much larger, long-term, comprehensive taxonomic study of the entire genus (PBI *Solanum*: a worldwide treatment; Knapp & al., 2004).

Molecular methods

Sequences of the *trn*T-F region, including the *trn*T-L and *trn*L-F intergeneric spacer regions (Taberlet & al., 1991), and the *waxy* gene (van der Leij & al., 1991; Mason-Gamer & al., 1998) were obtained for 40 *Solanum* and three outgroup species using standard DNA extraction, PCR, and sequencing methods described elsewhere (Bohs, 2004; Levin & al., 2005, 2006). The dense sampling within the *Leptostemonum* clade in (Levin & al., 2006) served as the framework for directed sampling of the representative old and new world *Solanum* species in this study. *Capsicum baccatum*, *Lycianthes heteroclita*, and *Jaltomata procumbens* served as outgroups.

Sequence alignment and analysis

Sequence alignment for *trn*T-F and *waxy* exons was straightforward and was performed manually using Se-Al (Rambaut, 1996). Although the *waxy* intron sequence alignment was more challenging, clearly recognizable sequence motifs that facilitated alignment were identified across all taxa. Similarly, most *trn*T-L spacer and *trn*L intron regions could be aligned with confidence. However, numerous sequence duplications have occurred in the *trn*L-F spacer between the 3' *trn*L and *trn*F exons within the species surveyed,

Table 1. Taxa studied, localities, vouchers with the herbaria in which they are deposited, and GenBank accession numbers. BIRM samples have the seed accession number of the Solanaceae collection at the University of Birmingham, UK. NIJ samples have accession numbers from the Solanaceae collection at the University of Nijmegen, Netherlands. Herbaria abbreviations are after the Index Herbariorum: Holmgren P.K., Holmgren N.H., New York Botanical Garden, http://sciweb.nybg.org/science2/IndexHerbariorum.asp.

Taxon	Locality	Vouchers	GenBank accession numbers	
			*trn*T-F	*waxy*
S. *abutiloides* (Griseb.) Bitter & Lillo	BIRM S.0655	Olmstead S-73 WTU	AY266236	AY562948
S. aethiopicum L.	BIRM S.0344	Olmstead S-74 WTU	DQ180394	AY996378
S. appendiculatum Dunal	Mexico	Anderson 1401 CONN	DQ180461	DQ169018
S. arboreum Dunal	Costa Rica	Bohs 2521 UT	DQ180424	AY996381
S. argentinum Bitter & Lillo	Argentina	Bohs 2539 UT	DQ180425	AY996382
S. aviculare Forst. f.	BIRM S.0809	no voucher	AY562952	AY559238
S. betaceum Cav.	Bolivia	Bohs 2468 UT	DQ180426	AY996387
S. candidum Lindl.	Costa Rica	Bohs 2898 UT	AY266237	AY562953
S. capense L.	NIJ 904750116	Bohs 2905 UT	DQ392958	AY996391
S. carolinense L.	BIRM S.1816	Olmstead S-77 WTU	DQ180476	AY996392
S. citrullifolium A. Braun	BIRM S.0127	Olmstead S-79 WTU	DQ180477	AY996395
S. cordovense Sessé & Moc.	Costa Rica	Bohs 2693 UT	DQ180480	AY996401
S. crinitipes Dunal	Colombia	Olmstead S-81 WTU	DQ180481	AY996402
S. cyaneo-purpureum De Wild.	NIJ 874750010	Bohs 3164 UT	DQ392959	AY996405
S. dulcamara L.	Cult. Michigan	no voucher	AY266231	AY996410
S. elaeagnifolium Cav.	Paraguay	Bohs 3204 UT (*trn*T-F) Bohs 3199 UT (*waxy*)	DQ180399	AY996412
S. herculeum Bohs	Morocco	Jury 13742 RNG	DQ180466	DQ169031
S. hindsianum Benth.	Mexico	Bohs 2975 UT	DQ180402	AY996424
S. jamaicense Mill.	BIRM S.1209	Olmstead S-85 WTU	DQ180485	AY562956
S. laciniatum Ait.	New Zealand	Bohs 2528 UT	DQ180467	AY996431
S. lidii Sunding	NIJ 934750022	Bohs 2903 UT	DQ180403	AY996434
S. luteoalbum Pers.	BIRM S.0042	Bohs 2337 UT	DQ180433	AY562957
S. lycopersicum L.	USA (cultivated)	no voucher	DQ180450	DQ169036
S. macrocarpon L.	BIRM S.0133	Olmstead S-88 WTU	DQ180404	AY996436
S. mammosum L.	BIRM S.0983	Olmstead S-89 WTU	AY266232	AY562958
S. myoxotrichum Baker	Madagascar	Bohs 2981 UT	DQ392960	AY996445
S. melongena L.	BIRM S.0657	Olmstead S-91 WTU	DQ180406	AY562959
S. nitidum Ruiz & Pav.	Bolivia	Nee 31944 NY	DQ180451	DQ169039
S. physalifolium var. *nitidibaccatum* (Bitter)	USA	Bohs 2467 UT	U47421	DQ169041
S. pseudocapsicum L.	BIRM S.0870	no voucher	DQ180436	AY562963
S. ptychanthum Dunal	USA	Olmstead S-94 WTU	DQ180454	AY996457
S. pyracanthos Lam.	USA (cultivated)	Olmstead S-95 WTU	DQ180408	AY996459
S. rostratum Dunal	USA	no voucher	DQ180489	AY996463
S. tomentosum L.	NIJ 894750127	Bohs 3107 UT	DQ392961	AY996473
S. torvum Sw.	BIRM S.0839	Olmstead S-101 WTU	AY266246	AY562972
S. tridynamum Dunal	BIRM S.1831	Olmstead S-102 WTU	DQ180412	AY996474
S. trisectum Dunal	France	Bohs 2718 UT	DQ180471	AY996475
S. vespertilio Aiton subsp. *vespertilio*	BIRM S.2091	Olmstead S-103 WTU	DQ180413	AY996476
S. wendlandii Hook. f.	BIRM S.0488	no voucher	DQ180440	AY562974
Capsicum baccatum var. *pendulum* (Willd.) Eshbaugh	USA (cultivated)	Eshbaugh 1584 MU	DQ180415	DQ169007
Jaltomata procumbens (Cav.) J.L. Gentry	Mexico	Davis 1189A	DQ180419	AY996374
Lycianthes heteroclita (Sendtn.) Bitter	Costa Rica	Bohs 2376 UT	DQ180414	DQ169010

and alignment in this region was highly ambiguous. The 3' *trn*L exon and the 432 aligned nucleotides of sequence data following were included in analyses; downstream sequences were excluded because they could not be aligned reliably.

Parsimony analyses were performed on each data set separately using PAUP*4.0b10 (Swofford, 2002). All characters were weighted equally in analyses implementing TBR branch swapping with 1,000 heuristic random addition replicates. Bootstrapping (Felsenstein, 1985) was used to evaluate branch support with 1,000 random addition replicates and TBR branch swapping. Prior to combining data sets, congruence was tested using the incongruence length difference test (Farris & al., 1994), implemented as the partition homogeneity test in PAUP*. One thousand partition homogeneity replicates were conducted, each with five random additions and TBR branch swapping. Analyses of the combined data followed the methods described in separate analyses.

Results

The *waxy* sequences ranged from 1,622 to 1,725 bases in length. Aligned sequence length was 1,811, and the alignment contained 333 parsimony informative characters. The four most parsimonious trees were of 1,108 steps, CI = 0.795, RI = 0.833. The length of *trn*T-F sequences was between 1,666 and 2,154 bases, with an aligned length (after excluding the 3 = sequence region) of 2,122 characters, of which 135 were parsimony informative. The 137,067 most parsimonious trees had a length of 434 steps, CI = 0.846, RI = 0.825.

Consistent with results of prior comprehensive studies (Levin & Miller, 2005; Levin & al., 2005, 2006), the ILD identified significant incongruence between the *waxy* and *trn*T-F partitions (p = 0.001). However, the ILD test is known to be prone to type I errors (Cunningham, 1997; Yoder & al., 2001) and is sensitive to variation in nucleotide substitution rates (Barker & Lutzoni, 2002). Because the *waxy* phylogeny is more resolved than the *trn*T-F phylogeny, we suspect differences in substitution rates between data sets may be the cause of the incongruence. Tree topologies of separate analyses were inspected visually. Topological differences did not have strong bootstrap support values (>90%), so we combined the data sets following the methods of Wiens (1998).

Trees inferred from the combined data were more resolved at all taxonomic levels than were those based on the separate analyses (Fig. 2). Analysis of the combined data produced 21 most parsimonious trees of 1,557 steps, CI = 0.802, RI = 0.823. *Solanum lidii* and *S. vespertilio* subsp. *vespertilio* were resolved as sister species (89% bootstrap support), nested well within the *Leptostemonum* clade (Fig. 2). The closest relatives of *S. lidii* and *S. vespertilio* subsp. *vespertilio* all are clearly of African origin. We also plotted some of the most unusual reproductive features on the cladogram (Fig. 2). The distribution of the characters fits expectations, but for some of them, more detailed field and greenhouse studies are needed to be certain of expression. For instance, the descriptions in Whalen (1984), Jaeger (1985), or in any taxonomic treatment that is of necessity based on herbarium specimens, are not always adequate to determine such things as weak andromonoecy or enantiostyly. Nonetheless, it is clear that with the exception of andromonoecy, the African representative species we included do not display heterandry, corolla zygomorphy or enantiostyly. The Madeiran *S. trisectum* is both heterandrous and zygomorphic, but it is also located at the base of the cladogram, phylogenetically distant from *S. lidii* and *S. vespertilio*.

Discussion

The cladogram (Fig. 2) clearly shows that the most obvious biogeographic connection, that to the closest continental area (Africa), is strongly supported by the molecular phylogenetic data as well. All of the most closely related species to the two Canarian *Solanum* examined are African, thus coinciding with previous molecular studies that inferred similar biogeographical connections for these taxa (Olmstead & Palmer, 1997; Levin & al., 2006). Not surprisingly, molecular studies on other African-Canarian species (reviewed by Andrus & al., 2004), have also suggested links between Macaronesia and regions of Africa.

Enantiostyly and zygomorphy (both in the corolla and staminal whorl) present in *S. vespertilio* and *S. lidii* suggested a connection with taxa in Mexico, hence the placement by Whalen (1984) of Canarian solanums in the otherwise Mesoamerican *Solanum* sect. *Nycterium*. Given the new phylogenetic understanding confirmed here, the characters shared between the Mexican and Canarian species do not constitute synapomorphies, but are instead parallelisms. The parallel development of the striking heterandrous condition and other associated features thus led to a taxonomic misinterpretation. The taxonomic impact of this kind of parallelism was previously demonstrated in other species including solanums (Anderson & al., 2002). For example, at the species level, the characters apparently linking a pair of taxa (*S. canense*

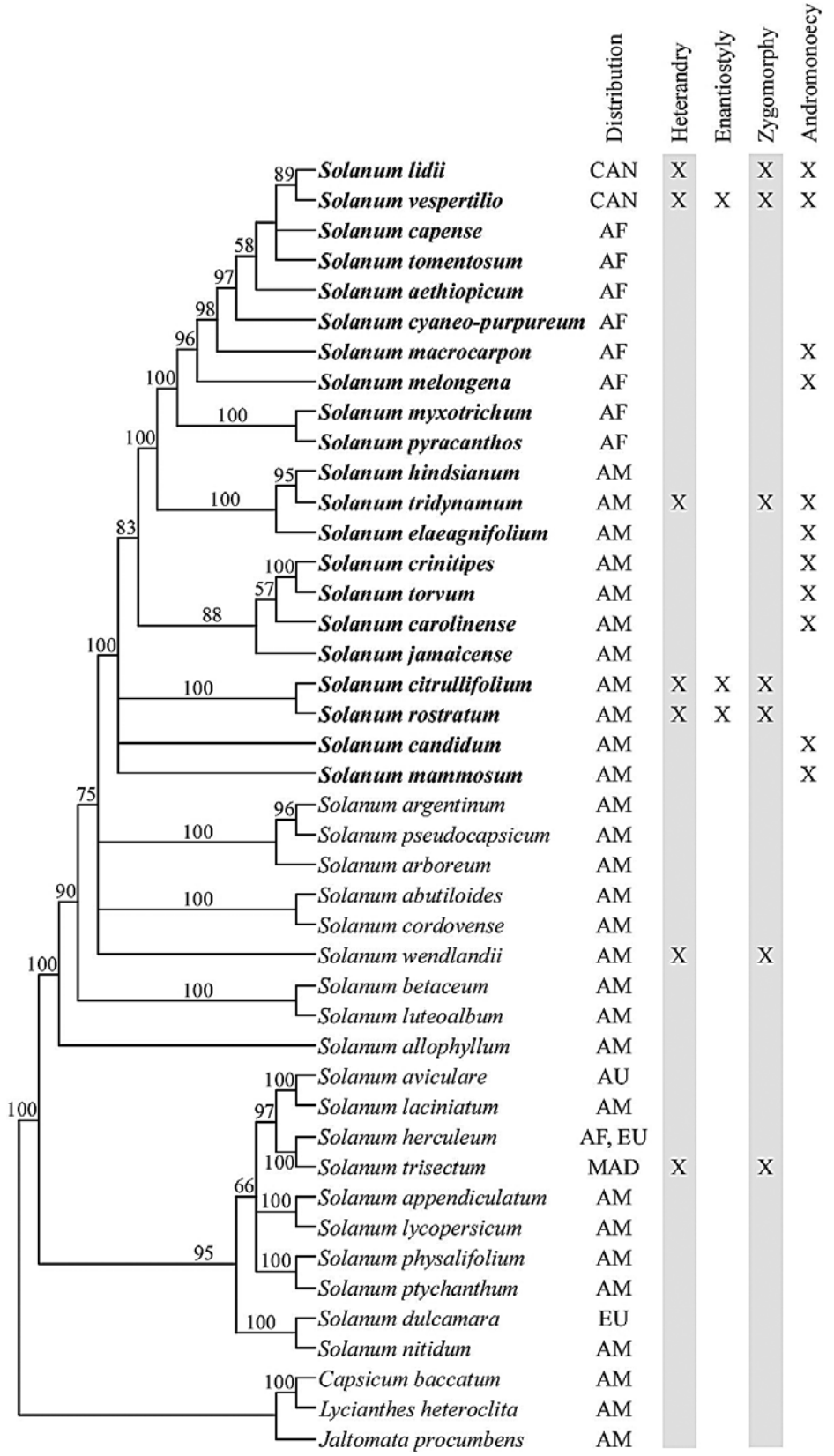

Fig. 2. The bootstrap consensus of the 21 most parsimonious trees for the combined analysis of the *trn*T-F and *waxy* sequence data sets. Bootstrap values ≥50% are shown above the branches. Bold indicates species from *Solanum* subgenus Leptostemonum. Geographical distribution of the species is abbreviated as follows: AF = Africa, AM = America, AU = Australia, CAN = Canary Islands, EU = Europe, MAD = Madeira. Reproductive characters are plotted with an X indicating presence and an empty box absence.

and *S. suaveolens*) were shown in molecular phylogenetic studies to be parallelisms associated with self compatibility and autogamy (Anderson & Jansen, 1998). And, in an even more striking case, Anderson & Symon (1989) proposed an underlying genetic structure in *Solanum* allowing expression of certain reproductive features in the right ecological context. Specifically, solanums from two continents (one species from Mexico/Guatemala [Anderson, 1979] and about 10 from Australia) have morphologically hermaphroditic flowers that are functionally dioecious. The pistillate flowers from both areas also include inaperturate pollen otherwise virtually absent in *Solanum* (inaperturate pollen also reported from the Ecuadorian *S. confertiseriatum*; Knapp & al. 1998). In this inter-continent comparison, there is no doubt that the expression of similar character suites is bound to represent a parallelism, also given that the species considered are in highly distinct phylogenetic groups (Spooner & al., 1993; Olmstead & Palmer, 1997; Knapp & al., 1998).

Likewise, the similarity of the Canarian and Mexican species we studied constitutes a parallelism: there is presumably the same genetic potential present for expression of heterandry and andromonoecy among these geographically and taxonomically distinct species. Furthermore, strong evidence is emerging for *Solanum* in general that indicates that features like zygomorphy, and even tetramery (for calyxes, corollas and stamens), are also often not synapomorphies, but instead, parallelisms, "underlying apomorphies", that have arisen independently a number of times (Levin & al., 2006).

The distribution of the reproductive features plotted on Fig. 2 suggests that a more complete taxon sampling of the approximately 80 native African *Solanum* species (Jaeger, 1985) would make projections on the evolution of some of the reproductive features of the Canarian taxa more sound. Some (~ 15-20%) of the native African species are reported as andromonoecious (Jaeger, 1985), though careful field and greenhouse studies are needed to clarify the perhaps even wider distribution of weak andromonoecy, a syndrome that is difficult to assess from herbarium specimens alone. None of the African species we included herein bear zygomorphic corollas (nor are there any in Jaeger's 1985 monograph). Three of the 80 native African species Jaeger includes have 'unequal anthers' (staminal 'zygomorphy'), but all are presently distributed in northeast to east-coastal Africa (Jaeger, 1985), a continent away from the Canaries. Jaeger (1985) does not record any enantiostylous species for Africa, but that character would be especially hard to detect in an herbarium-based study. However, more significantly, there are no citations of enantiostyly for African solanums in the comprehensive work on enantiostyly by Jesson and colleagues (Jesson & Barrett, 2003, 2005; Jesson & al., 2003).

This character distribution would imply that it is reasonable to consider the evolution of at least some of the reproductive features of this interesting pair of rare Canary Island endemics must be considered *in situ*. Given the possibility that the colonizing forms might have been andromonoecious, but likely were not heterandrous, zygomorphic or enantiostylous, it is the biological and physical components of the Canary Islands, including the suite of available pollinators, that we must look at in the context of the possible African progenitors to understand the evolution of the reproductive elements.

As pointed out by Carlquist (1974) and others, isolation to islands can lead to a release from selection and establishment of unusual characters. It remains to be demonstrated that the island setting *per se* is associated with the reproductive features present in the Canarian solanums. It is of some interest that all four of the endemic Solanaceae on the Canary islands express notable reproductive features for the genus or family. The other known species of *Solanum* from this archipelago is "new." *Solanum nava* was recently placed in the genus as a result of the transfer from *Normania* following molecular phylogenetic work (Bohs & Olmstead, 2001). Actually, *S. nava* was originally placed in *Solanum* when it was first described. It got transferred to *Normania* and now is back in *Solanum. Solanum nava* is only very distantly related to *S. vespertilio* and *S. lidii*, and in fact is nearly basal in *Solanum*. However, it too has zygomorphic flowers, though the zygomorphy is much less pronounced than in *S. vespertilio* or *S. lidii*. Furthermore, *S. nava* is also heterandrous (Francisco-Ortega & al., 1993; Bohs & Olmstead, 2001), but the manifestation of stamen heteromorphy is very different. The androecium in *S. nava* consists of two long curved anthers, two shorter curved anthers, and one very short anther, not curved. The four longer anthers differ as well in having a projection or horn at the middle or near the base (Francisco-Ortega & al., 1993; Carrizo García, 2001). The case of *S. nava* also differs in that it is possible that the ancestor of *S. nava* may at least have had a zygomorphic corolla (if not heterandrous), given that the closest relatives of this Canarian species are the Madeiran species *S. trisectum* (also zygomorphic) and the weakly zygomorphic *S. herculeum* (formerly *Triguera osbeckii*, from southern Spain and northwest Africa; Bohs & Olmstead, 2001). Finally, the other en-

demic member of the Solanaceae on the Canary Islands is *Withania aristata*. Anderson and colleagues (2006) have recently shown this species to be dioecious, although it was previously widely treated as bearing hermaphroditic flowers (e.g., Bramwell & Bramwell, 2001).

The molecular phylogenetic data presented here, and the karyotypic data elsewhere (Chiarini & al., 2006) do not address the question of sympatric, parallel, or sequential speciation. However, studies of genetic variation of *S. vespertilio* and *S. lidii* using AFLPs do (Prohens & al., in press). These studies by Prohens and colleagues show that *S. lidii*, endemic to Gran Canaria, is genetically closer to putative African relatives than *S. vespertilio*, which is largely restricted to Tenerife. Since Gran Canaria is older than Tenerife (Coello & al., 1992), it is possible that the speciation event that gave rise to *S. vespertilio* was more recent than that for *S. lidii. Solanum vespertilio* (vs. *S. lidii*) also possesses a corolla that shows much more pronounced zygomorphy, is tetramerous (vs. pentamerous), and some of its flowers express enantiostyly. These differences support the hypothesis of *in situ* development of the reproductive features, or at least some of them, on the Islands. Interestingly, the three endemic solanums in the Hawaian archipelago all bear "distinctly incurved anthers", a circumstance that D. Symon suggests may be related to "a distinctive pollinator" (Wagner & al., 1990). On the other hand, not all island solanums are 'distinctive' in this context: the lone endemic *Solanum* to the Juan Fernandez islands, *S. fernandezianum*, is highly autogamous, but bears typical 'solanum' flowers (Ramanna & Hermsen, 1981).

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